clathrin heavy chain. This self-assembling fragment is the central portion or Hub of the three-legged clathrin triskelion. Previous studies have suggested that assembly interactions between Hub legs display micromolar affinity and likely involve hydrophobic interactions, though assembly is modulated by a pH-sensitive salt bridge. The studies to be reported investigated whether mid-infrared spectroscopy of Hubs in solutions and also in a controlled humidity environment can be used to establish additional features of Hub self-assembly and potentially as a dynamic monitor of clathrin assembly. Comparison of spectra generated from assembled and disassembled clathrin revealed that hydration plays a role in assembly and that several absorption bands (1117 and 1220 wavenumbers) were present in assembled hubs that were absent in unassembled hubs. Such spectra were obtained on a Bruker 66v/S. Spectra generated during assembly suggested that a decrease in random coil and an increase in alpha helical content occur during Hub assembly, indicative of increased thermodynamic stability achieved during lattice formation. (Preliminary Raman data was also obtained from assembled Hubs.) These results demonstrate that analysis of Hub behavior in the infrared can be informative about the dynamics of clathrin self-assembly and suggest infrared spectroscopy as a novel approach to understanding the molecular details of clathrin-coated vesicle formation.

**2597-Pos**

**Internalization of Two Distinct Receptors in Response to Occupation with a Bivalent Ligand Incorporating a Single Stimulus for Internalization**

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Treatment of patients with CCK2 receptor antagonists potentiates pain relief induced by mu-opioid (MOP) agonists. In attempt to enhance this effect with a single bivalent ligand, we connected pharmacophores of non-peptidyl CCK2 receptor antagonist and MOP receptor agonist with a spacer. Spacer length of 16-21 atoms was consistent with simultaneous binding to both receptors, however provided no advantage in biological activity from that of two individual ligands (J Med Chem, 2009). Here, we extend this to examine effects of ligand tethering on receptor regulation. We prepared a series of CHO cell lines stably expressing yellow fluorescent protein (YFP)-tagged single receptor constructs or both of these receptors tagged with half of YFP (YN attached to one receptor and YC attached to the other). The YFP halves were not fluorescent until brought into spatial approximation to reconstitute YFP. These receptors bound their specific ligands effectively. The MOP agonist signaled normally and resulted in MOP receptor internalization. The CCK2 receptor antagonist did not stimulate receptor internalization. In the dual receptor-expressing cell line, bivalent ligands capable of binding both receptors simultaneously effected YFP fluorescence at the cell surface, and this signal internalized in a time- and temperature-dependent manner. Bivalent ligands with spacer arms too short to occupy both receptors simultaneously did not result in such a signal. Thus, a bivalent ligand is able to stimulate the association of two non-spontaneously-dimerizing receptors on the cell surface, and both of these receptors are internalized in response to binding a ligand of one receptor that stimulates internalization. Tethering provides a mechanism for dragging other surface molecules into the endocytic pathway.

**2598-Pos**

**Vesicular Monoamine and Glutamate Transporters Select Distinct Synaptic Vesicle Recycling Pathways**

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Monoamine neurotransmitters including dopamine, norepinephrine and serotonin are involved in a number of vital functions, including the control of movement, attention, motivation, emotional state, learning, and memory. The role of dopamine in reward requires the release of more dopamine in response to reward-relevant burst firing than to the single action potentials of background pacemaking activity. We have developed a new reporter, VMAT2-pHluorin to follow vesicular recycling required for dopamine release. We used the vesicular monoamine transporter VMAT2 and the vesicular glutamate transporter VGLUT1 to compare the localization and recycling of synaptic vesicles that store monoamines and glutamate, and observed several surprising differences. First, VMAT2 segregates partially from VGLUT1 in the dopaminergic synapses, but not in glutamatergic neurons. Second, post- stimulus endocytosis is slower for VMAT2 than VGLUT1 in both cell populations. During the stimulus, however, the endocytosis of VMAT2 accelerates dramatically in dopamine neurons, indicating a novel mechanism to sustain high rates of release. Furthermore, we find that in both cell types, a substantially smaller proportion of VMAT2 than VGLUT1 is available for evoked release. VMAT2 also shows considerably more dispersion along the processes after exocytosis than VGLUT1. Even when expressed in the same cell type, the vesicular transporters select distinct pathways for the recycling of synaptic vesicles that release dopamine and glutamate.

**2599-Pos**

**Uptake by Human Astrocytes of Lipid Vesicles Modeling the Lipid Composition of Myelin**

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The myelin sheath is composed of around 80% lipids including cholesterol, phospholipids, sphingomyelin, cerebroside sulfate and cerebrosides, and in smaller proportions, ceramides and glycerophosphatides. During pathological processes inside the central nervous system (CNS), the damage of this axonal insulation may expose the surrounding glial cells to lipid aggregates that result from this demyelination process. Indeed, microglia, considered to function as the local macrophages, can phagocyte myelin and cell detritus. Human astrocytes are another key example of glial cells where uptake of myelin debris may take place. Astrocytes are key regulators of several neuronal protective mechanisms, but they are also involved in the pathogenesis of certain autoimmune and inflammatory CNS diseases. Aiming to probe the behavior of human astrocytes exposed to different myelin lipids, we monitor the dynamics of lipid vesicle uptake by culture cells, and explore how varying specific myelin lipid components regulate the uptake kinetics and cell viability. A human astrocyte cell line obtained from a glioblastoma is used for all the experiments. Cells are exposed to NBD-PE or calcein labeled 50 nm small unilamellar vesicles (SUVs) of various lipid compositions reflecting various combinations of the myelin lipid components. Vesicle uptake is then monitored through fluorescence spectroscopy at different time points. Significant uptake is observed within 30 minutes, reaching saturation levels around 2 hours. These results are corroborated through flow cytometry, where astrocyte fluorescence stabilizes at around 2 hours. Additionally, we observe the presence of a smaller population of scatter cells thatshowed higher liposome uptake. Finally, by using fluorescence/DIC microscopy, liposomes are found to spread in the astrocyte cytoplasm after 4 hours of incubations. Interestingly, crowding of liposomes around the nucleus is observed after 12 hours of incubation, suggesting a sorting mechanism to be determined.

**2600-Pos**

**Atomistic and Continuum Modeling of Cellular Uptake of Nanotubes and Viruses**

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Most viruses and bioparticles endocytosed by cells have characteristic sizes in the range of tens to hundreds of nanometers. Recent experimental observations have shown that nanoparticles such as carbon nanotubes (CNT) can enter animal cells. The process of viruses and nanoparticles entering and leaving animal cells is thought to be mediated by the binding interaction between ligand molecules on the viral capid and their receptor molecules on the cell membrane. Here we conduct coarse grained molecular dynamics and theoretical studies of the intrinsic interaction mechanisms of nanoparticles and viruses of different shapes and sizes with a lipid bilayer [1-3]. Theoretical models are proposed to explain the observed size and shape effects in various entry mechanisms.

Selected References:

[1] X. Shi, Y. Kong and H. Gao, "Coarse grained molecular dynamics and theoretical studies of carbon nanotubes entering cell membrane," 2008, Acta Mechanica Sinica, Vol. 24, pp. 161-169.
[2] H. Gao, W. Shi and L. B. Freund, "Mechanics of receptor-mediated endocytosis," 2005, Proceedings of the National Academy of Sciences of USA, Vol. 102 (27), pp. 9469-9474.
[3] W. Shi, J. Wang, X. Fan, and H. Gao, "Size and shape effects on diffusion and absorption of colloidal particles near a partially absorbing sphere: Implications for uptake of nanoparticles in animal cells," 2008, *Physical Review E*, **Vol**. **78**(12), Art. No. 061914.